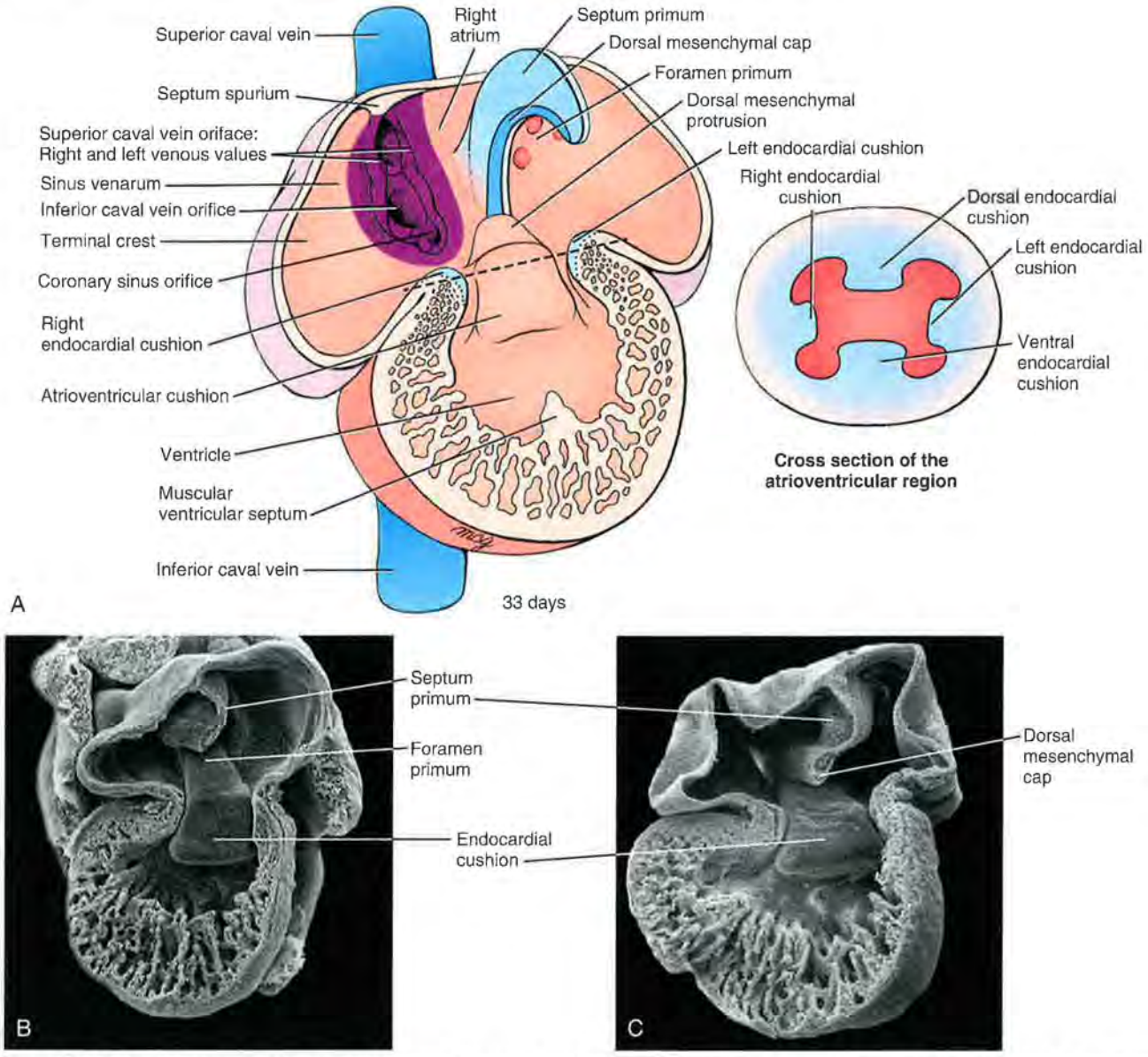

Figure 12-21. Initial septation of the atria. *A,* The septum primum and the dorsal mesenchymal protrusion form on the dorsal roof of the atrial chamber during the fifth week and grow together toward the atrioventricular canal. A ridge of endocardium-derived mesenchymal cells, called the dorsal mesenchymal cap, develops along the rim of the septum primum. Simultaneously, the atrioventricular canal is divided into right and left atrioventricular orifices by the growing dorsal and ventral endocardial cushions. Dashed line represents the level of the cross section through the atrioventricular canal region. *B, C,* Scanning electron micrographs show foramen primum and the developing septum primum and its dorsal mesenchymal cap.

On about day twenty-six, while atrial remodeling is ongoing, the roof of the atrium develops a depression along the midline at the site beneath the overlying outflow tract. On day twenty-eight, this deepening depression results in a crescent-shaped myocardial wedge, called the septum primum, which extends into the atrium from the cranial-dorsal wall as the primitive atrial chamber expands (Fig. 12-21*A*). On the leading edge of the septum primum, a mesenchyme-filled ridge called the **dorsal mesenchymal cap** is found, which, like the atrioventricular and outflow tract cushions, contains endocardially derived mesenchyme (Fig. 12-21*A-C*). Meanwhile at the venous pole, second heart field–derived cells project into the atrium using the dorsal mesocardium as a port-of-entry (see Fig. 12-19*B*). This cell population, called the **dorsal mesenchymal protrusion** (or **spina vestibuli**), is contiguous with the dorsal mesenchymal cap on the septum primum and the dorsal atrioventricular cushion (Figs. 12-21*A*, 12-22*A*).

As the septum primum elongates by differential growth, the dorsal and ventral atrioventricular cushions fuse to form the **atrioventricular septum** (or **septum intermedium**), thereby dividing the common atrioventricular orifice into separate **right** and **left atrioventricular canals** (see Fig. 12-22*A*). The dorsal mesenchymal cap, dorsal mesenchymal protrusion, and atrioventricular

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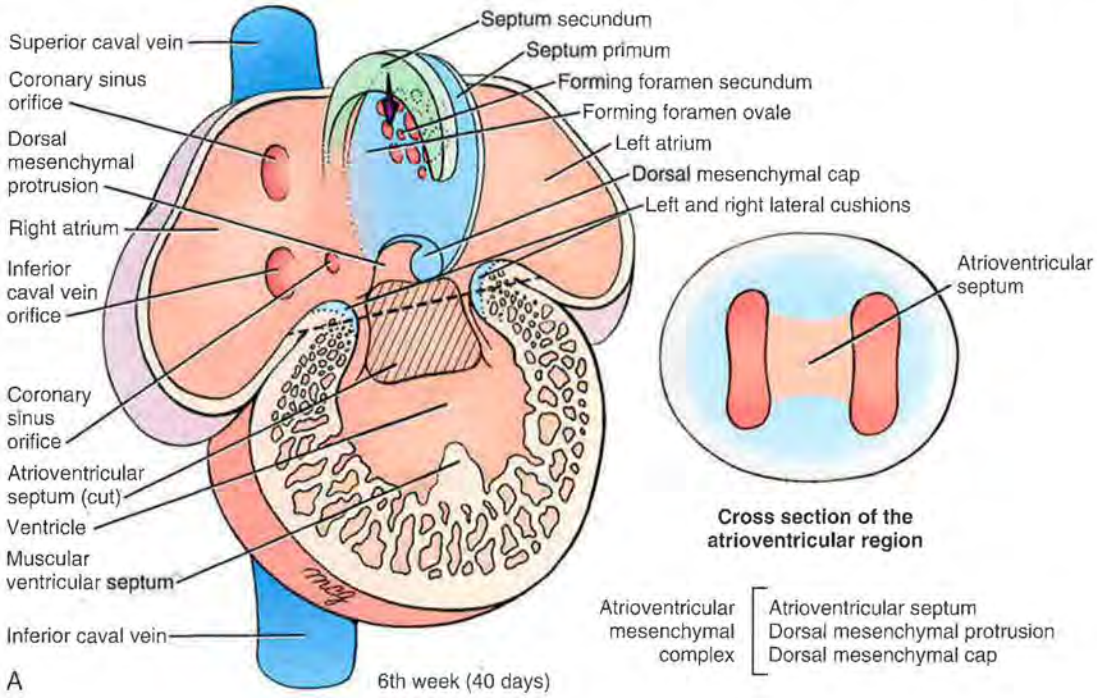

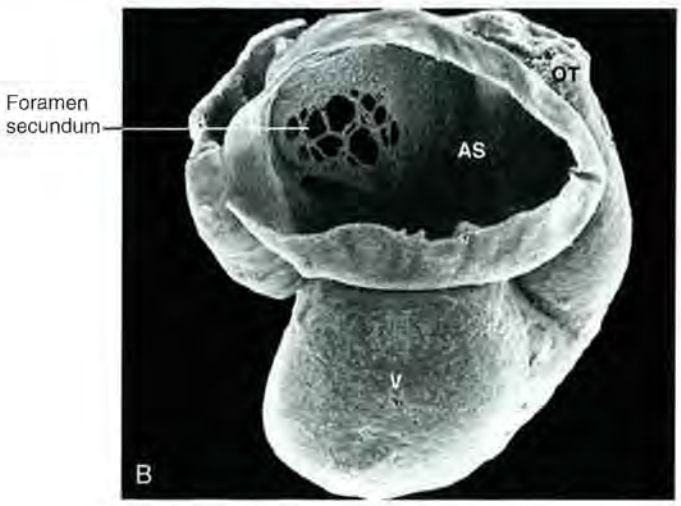

Figure 12-22. Further septation of the atria. *A,* During the sixth week, the thick septum secundum grows from the roof of the right atrium, and the septum primum, its dorsal mesenchymal cap, and the dorsal mesenchymal protrusion fuse with the atrioventricular cushion to fill the foramen primum. However, before the foramen primum is obliterated, the foramen secundum forms by the coalescence of small ruptures in the septum primum. Dashed line represents the level of the cross section through the atrioventricular canal region. *B,* Scanning electron micrograph showing the development of the foramen secundum. AS, Atrial septum; OT, outflow tract; V, ventricle.

septum then fuse to form the **atrioventricular mesenchymal complex**, filling the remaining interatrial connection **(foramen primum** or **ostium primum)** (see Figs. 12-21, 12-22*A*, 12-23). As the atrioventricular mesenchymal complex closes the foramen primum, programmed cell death in the dorsal region of the septum primum creates small perforations that coalesce to form a new foramen, the **foramen secundum** (or **ostium secundum**) (see Fig. 12-22*A, B*). Thus, a new channel for right-to-left shunting between atrial chambers opens before the old one closes.

While the septum primum is lengthening, a second crescent-shaped ridge of tissue forms on the ceiling of the right atrium, just adjacent to and to the right of the septum primum (see Fig. 12-22*A*). This **septum secundum** is thick and muscular, in contrast to the thin septum primum. The edge of the septum secundum grows cranial-caudally and dorsal-ventrally, but it halts before it reaches the atrioventricular mesenchymal complex, leaving an opening called the **foramen ovale** near the floor of the right atrium (see Figs. 12-22*A*, 12-23). Therefore, throughout the rest of fetal

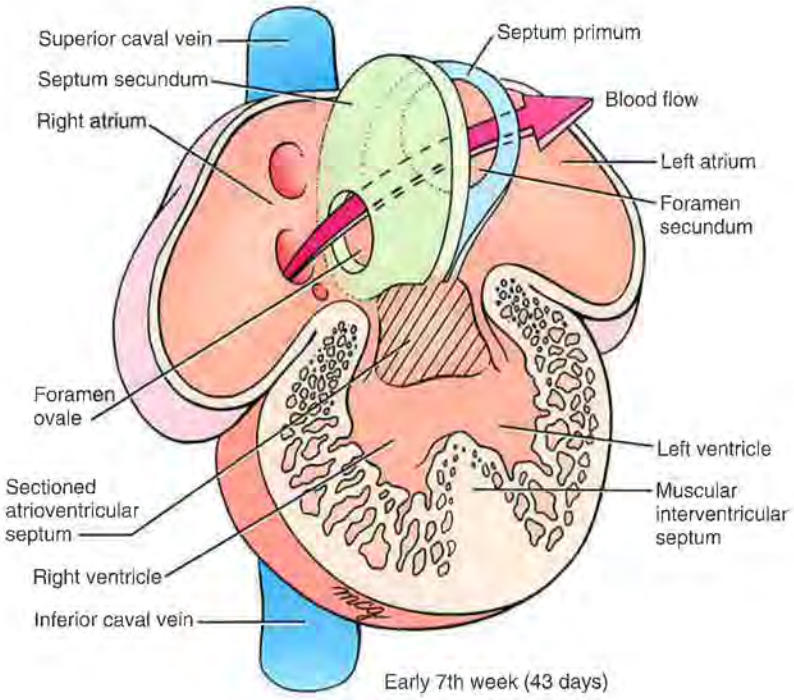

Figure 12-23. Definitive fetal septation of the atria. The septum secundum does not completely close, leaving an opening in this septum called the foramen ovale. During embryonic and fetal life, much of the blood entering the right atrium passes to the left atrium via the foramen ovale and the foramen secundum.

development, blood shunts from the right atrium to the left atrium and passes through the two staggered openings. This arrangement allows blood to flow from the right atrium to the left atrium, but not in the reverse direction, as the thin septum primum collapses against the stiff septum secundum, effectively blocking blood flow back into the right atrium. This shunt closes at birth because abrupt dilation of the pulmonary vasculature combined with cessation of umbilical flow reverses the pressure difference between the atria and pushes the flexible septum primum against the more rigid septum secundum, even during atrial diastole (see "Dramatic Changes Occur in Circulatory System at Birth," in Chapter 13).

REALIGNMENT OF PRIMITIVE CHAMBERS

Animation 12-6: Realignment of Heart Chambers.
Animations are available online at StudentConsult.

Even after cardiac looping is nearly finished, the atrioventricular canal provides a direct pathway only between the primitive atrium and the primitive left ventricle (Fig. 12-24*A*). Moreover, the proximal end of the primitive right ventricle, but not the primitive left ventricle, is initially continuous with the outflow tract, which will give rise to both aortic and pulmonary outflow vessels. Proper cardiac tube looping, chamber expansion, and realignment must occur to bring the developing atrioventricular canal into alignment with the right atrium and the right ventricle, and to provide the left ventricle with a direct path to the outflow tract. This process is illustrated in Figure 12-24.

The atrioventricular canal initially lies mainly between the primitive atrium and the primitive left ventricle. The mechanism by which the right and left atrioventricular canals come into alignment with the future right and left ventricles is unclear. However, this change may be accomplished by active remodeling of the **primary muscular fold**. Beneath the right (tricuspid) portion of the atrioventricular canal, a small slit forms in the myocardium of the **muscular ventricular septum**. This slit expands to form a proper right ventricular inflow tract, enabling the tricuspid orifice to become positioned above the right ventricle (Fig. 12-24*C*). At the same time, the left part of the common outflow tract becomes more associated with the left ventricle. Meanwhile, the dorsal and ventral atrioventricular cushions are growing, and by the time the common atrioventricular canal has split into right and left canals, the latter are correctly aligned with their respective atria and ventricles (see Fig. 12-24*C*).

Once the atrioventricular canals, ventricles, and cardiac outflow tract are all correctly aligned, the stage is set for the remaining phases of heart morphogenesis: completion of atrial septation, septation of the ventricles, septation of the outflow tract into ascending aorta and pulmonary trunk, and development of heart valves, coronary vasculature, and conduction system.

INITIATION OF SEPTATION OF VENTRICLES

Animation 12-7: Partitioning of Ventricle.
Animations are available online at StudentConsult.

At the end of the fourth week, the **muscular ventricular septum**, located between the presumptive right and left ventricular chambers, begins to become a more prominent structure as the ventricles are in the

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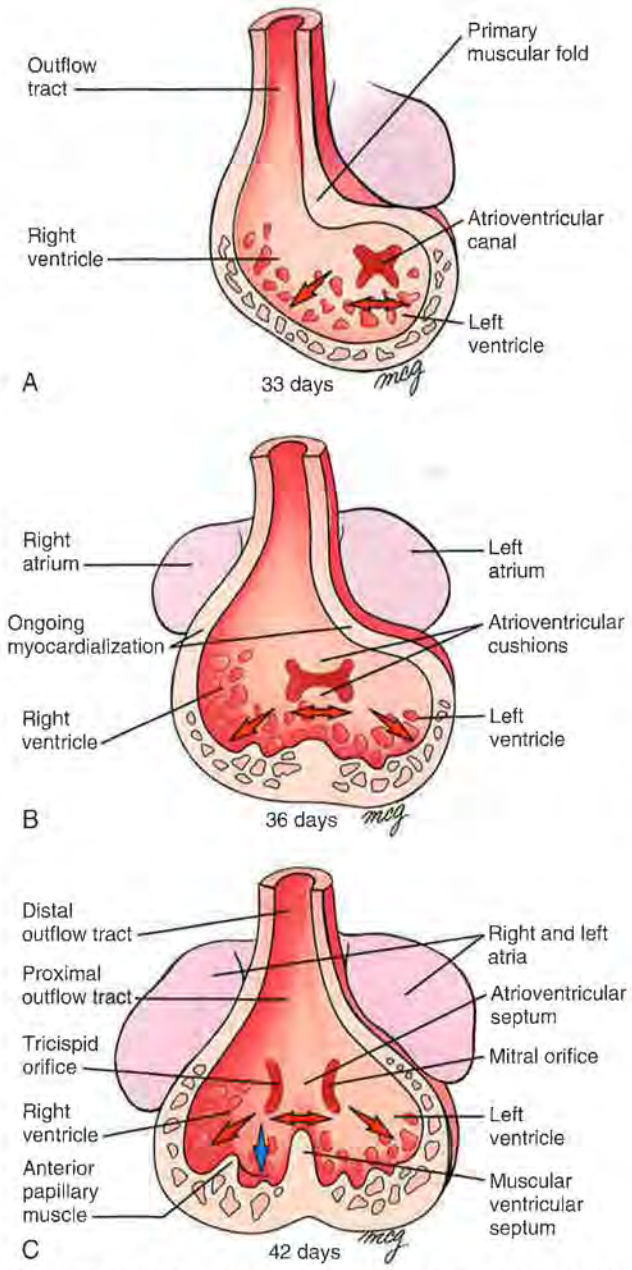

Figure 12-24. *A-C*, Realignment of the heart. As the atrioventricular septum forms during the fifth and sixth weeks, the heart is remodeled to align the developing left atrioventricular canal with the left atrium and ventricle, and the right atrioventricular canal with the right atrium and ventricle. Red arrows indicate the direction of realignment of the atrioventricular canal and outflow tract and formation of the muscular interventricular septum. The blue arrow in *C* indicates formation of an enlarging slit carved out of the muscular ventricular septum; this is responsible in part for repositioning of the tricuspid orifice to the right, as well as for formation of the moderator band.

process of expanding (Fig. 12-25; see also Fig. 12-24). Although the muscular ventricular septum continues to grow, closure of the **interventricular foramen (primary ventricular foramen)** does not occur until the eighth week of development. If fusion of the muscular ventricular septum with the atrioventricular mesenchymal complex would occur too soon, the left ventricle would be shut off from the ventricular outflow tract.

At the same time that the muscular ventricular septum is forming, the myocardium begins to thicken and myocardial ridges or **trabeculae** develop on the inner wall of both ventricles. Trabeculation begins at about the fourth week of development, with projections or ridges first forming in the outer curvature of the heart. These trabecular ridges are transformed into fenestrated trabecular sheets while the outer cardiomyocytes adjacent to the epicardium rapidly proliferate, forming an outer compact layer of myocardium (Figs. 12-25, 12-26).

On the right wall of the muscular ventricular septum, a prominent trabeculation called the **septomarginal trabeculation (moderator band)** develops. Owing to expansion of the right ventricular chamber inlet, the septomarginal trabeculation crosses the right lateral wall, extending toward the developing anterior papillary muscle of the tricuspid valve (see Fig. 12-24*C*). Expansion of the right ventricular chamber inlet drives formation of a large part of the mature right ventricular chamber. If expansion of this area is insufficient, the developing tricuspid portion of the atrioventricular canal can remain associated with the primary ventricular foramen, leading to tricuspid atresia and other valve anomalies.

In the Research Lab

Myocardium Develops Two Layers

As mentioned earlier, the myocardial wall develops two basic layers: an inner **trabecular layer** of myocardium and an outer **compact layer** of myocardium. The trabecular layer contributes to cardiac contraction and increases the inner surface area, thereby facilitating nutritional and gaseous exchange while the coronary vasculature is developing. Trabeculae grow from clonal expansion of myocardial cells, leading to the formation of these myocardial infoldings (see Figs. 12-25, 12-26). In mice, the Egf receptors ErbB2 and ErbB3 (expressed in the myocardium) and one of their ligands, neuregulin (expressed in the endocardium), are required for trabecular development, as well as for gestational survival. In humans, overproduction of trabeculae at the expense of the compact myocardial layer leads to **isolated ventricular non-compaction**, a condition that can cause sudden heart failure. Formation of the outer compact layer of myocardium requires an interaction with the developing epicardium. A thin compact layer of myocardium can be the result of deficiencies in the interaction between epicardium-derived cells and outer myocardial wall. For example, in mice null for the retinoic acid receptor, RXRα, proliferation of myocardial cells in the compact layer fails and the mice die early in utero. Retinoic acid receptors are expressed in epicardial cells; in response to retinoic acid, the epicardium releases Fgfs that stimulate myocardial cell proliferation. In the absence of retinoic acid signaling, cardiomyocytes prematurely differentiate and hypertrophy rather than proliferate first. These mice exhibit dilated cardiomyopathy at birth.

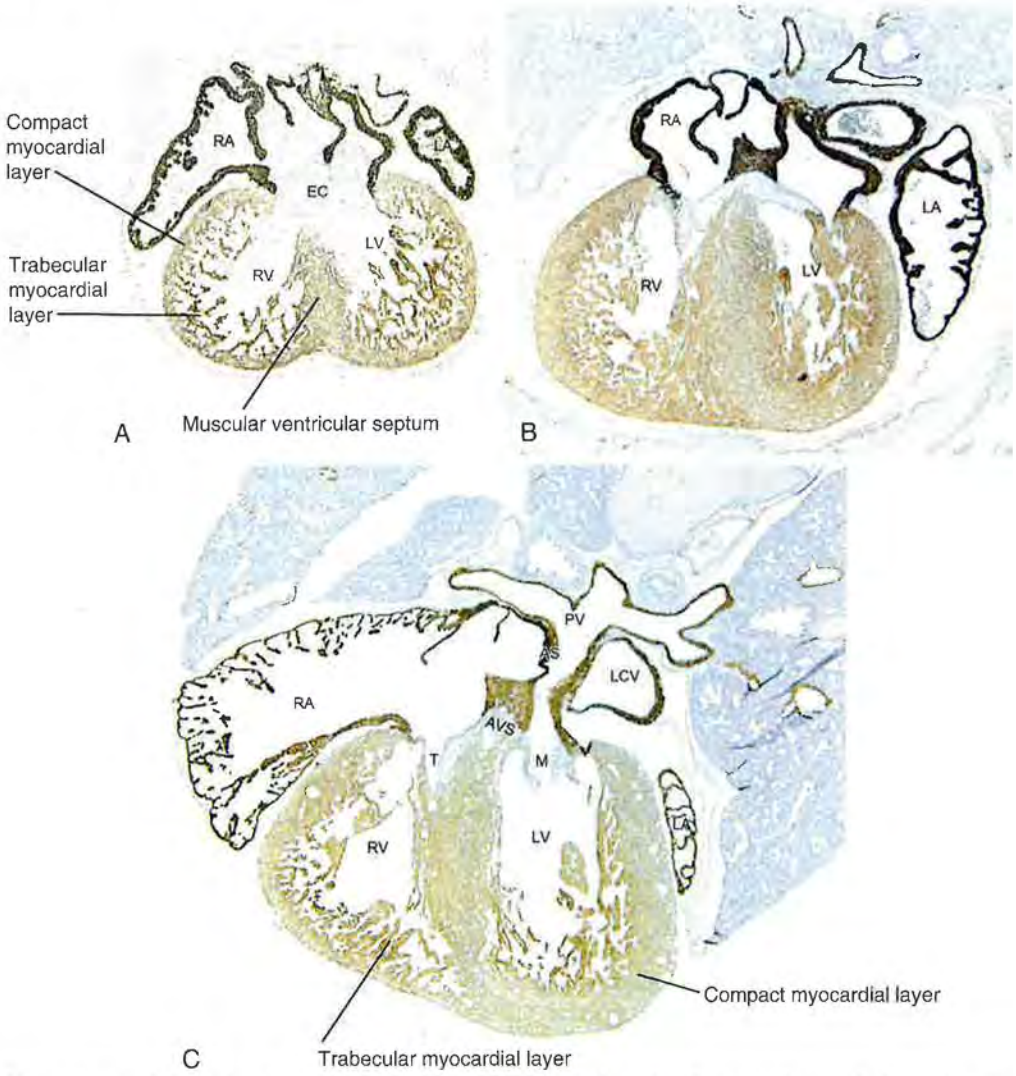

Figure 12-25. Photomicrographs showing the development of the atrioventricular valve, the muscular ventricular septum, and the trabecular and compact layers of myocardium in the developing mouse embryo. *A-C,* Tissue samples were immunostained with an antibody recognizing, a light chain atrial form of myosin expressed in both atria and ventricles at this stage of development. AS, Atrial septum; AVS, atrioventricular septum; EC, endocardial cushion tissues; LCV, left coronary vein; M and T, developing mitral and tricuspid valves, respectively; PV, pulmonary vein; RA and LA, right and left atria, respectively; RV and LV, right and left ventricles, respectively; VS, muscular ventricular septum.

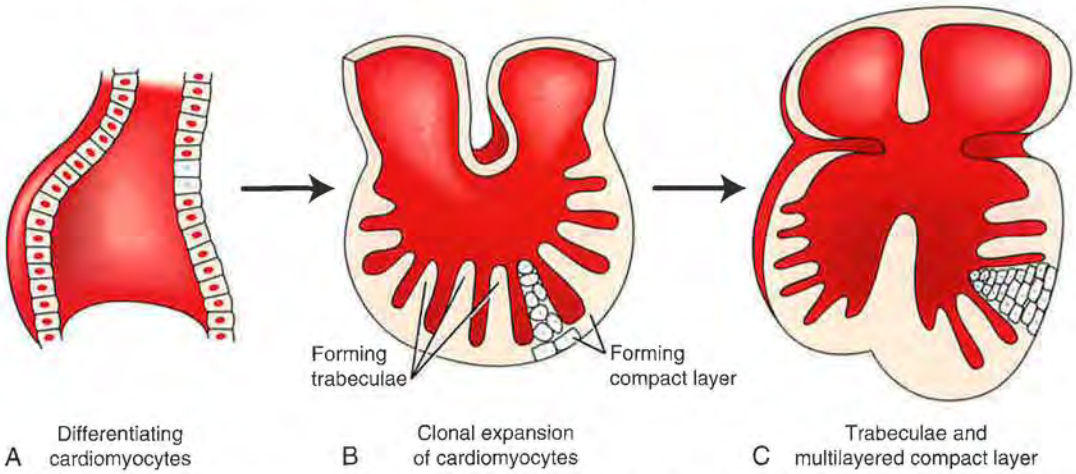

Figure 12-26. Formation of cardiac trabeculae. *A-C,* Myocardial trabeculae develop from clonal expansion of cardiomyocytes within the myocardial wall and are subsequently remodeled as the heart increases in size. Clonal expansion of the compact layer into a multilayered myocardium requires tissue-tissue interactions with the developing epicardium.

DEVELOPMENT OF ATRIOVENTRICULAR VALVES

Animation 12-8: Formation of AV Valves.
Animations are available online at StudentConsult.

The atrioventricular valves begin to form between the fifth and eighth weeks. These **valve leaflets** are firmly rooted in connective tissue (the **fibrous annulus**) surrounding the right and left atrioventricular canals and are thought to arise from proliferation and differentiation of the adjacent endocardial cushion tissues. How the mature valves are formed is not fully understood. Morphological and lineage tracing studies in several animal models show that the bulk of the leaflet cells are derived from endocardial cushion tissue with some contribution of cells coming from the epicardium (Fig. 12-27*A, B*). The leaflets are freed from the myocardial wall by remodeling and erosion of the ventricular myocardial wall. This leads to the formation of ventricular outpockets beneath the valve primordia and leaves thin strands of cells that form the **chordae tendineae** and small hillocks of myocardium called **papillary muscles** (Fig. 12-27*B, C*). The valve leaflets are designed so that they fold back to allow blood to enter the ventricles from the atria during diastole but shut to prevent backflow when the ventricles contract during systole. The left atrioventricular valve has only anterior and posterior leaflets and is called the **mitral (bicuspid) valve** The right atrioventricular valve usually (but not always) develops a third, small **septal cusp** during the third month; therefore, it is called the **tricuspid valve** (see Fig. 12-27*C*).

SEPTATION OF OUTFLOW TRACT AND COMPLETION OF VENTRICULAR SEPTATION

Animation 12-9: Partitioning of Outflow Tract.
Animations are available online at StudentConsult.

Animation 12-10: Formation of Membranous Interventricular Septum.
Animations are available online at StudentConsult.

When the muscular ventricular septum ceases to grow, the two ventricles still communicate with each other through the interventricular foramen (Fig. 12-28*A-C*). Separation of the outflow tract and ventricles must be coordinated with realignment of the outflow tract relative to the ventricles if the heart is to function properly. It is not surprising to note that a large proportion of cardiac

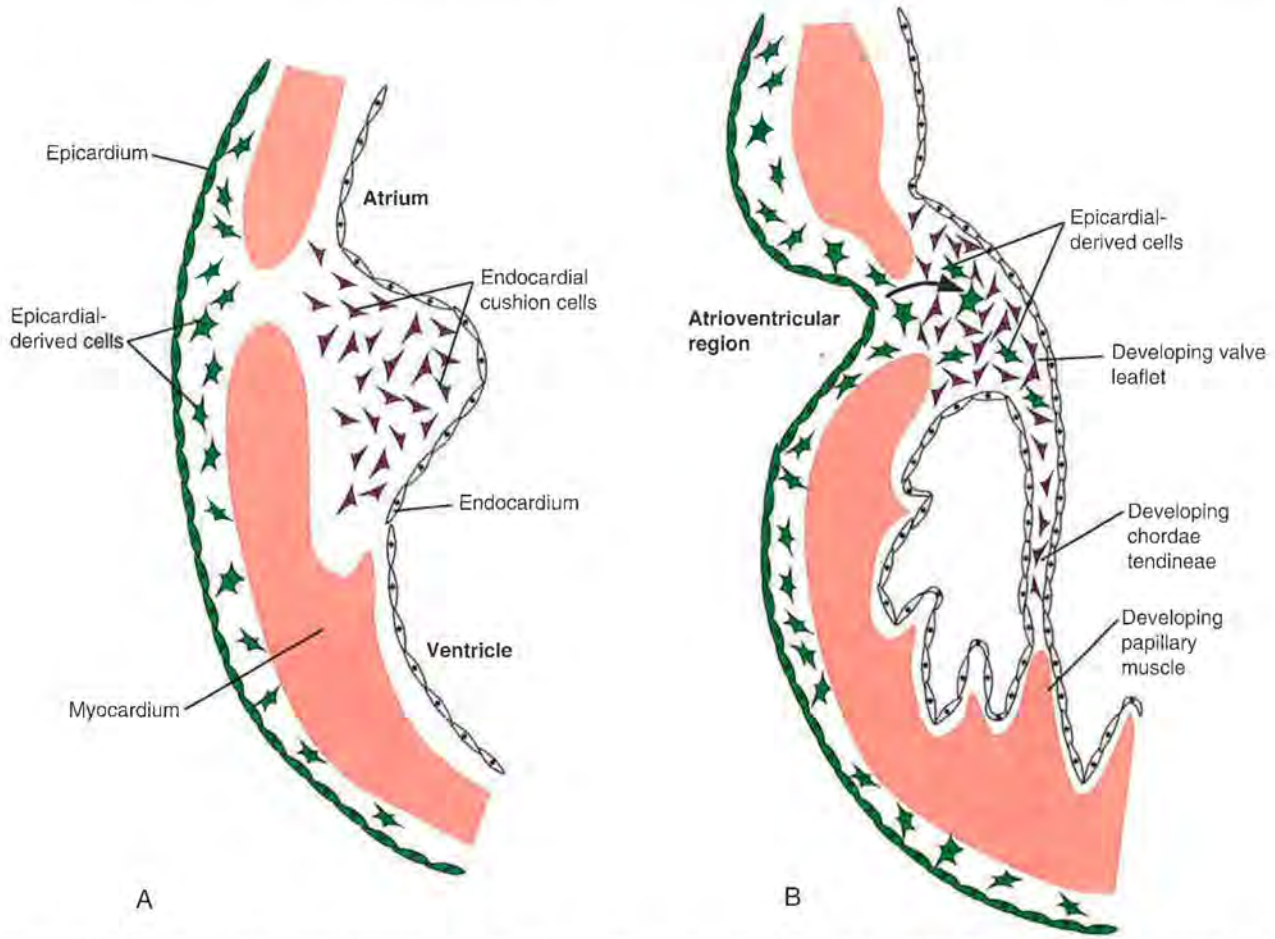

Figure 12-27. Development of the atrioventricular valves. *A, B,* Portion of the wall of the right ventricle at two stages in atrioventicular valve development. The structures composing the atrioventricular valves, including the papillary muscles, chordae tendineae, and cusps, are sculpted from the muscular walls of the ventricles. Valve leaflets are derived from endocardial cushion tissue, with some contribution from epicardium-derived cells entering along the margin of the atrioventricular region. Arrow in *B* indicates the direction of migration of these cells.

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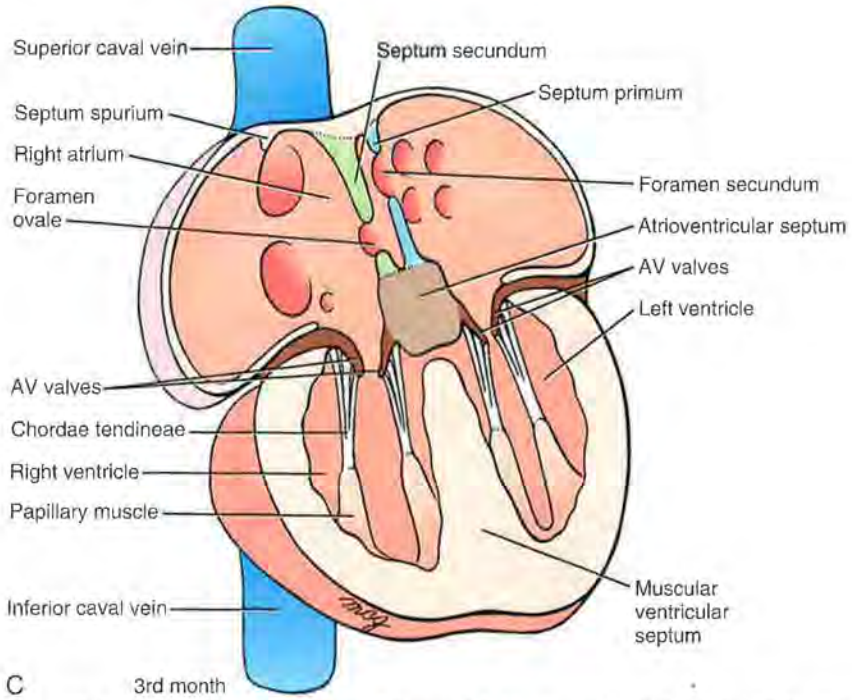

Figure 12-27, cont'd *C*, The definitive tricuspid valve within the right ventricle is not completely formed until the development of a septal cusp in the third month.

defects are the result of errors in this complex process (discussed later in the chapter).

Division of the cardiac outflow tract is complex and is not completely understood. The cardiac outflow tract is divided by the formation of a pair of **endocardium-derived outflow tract cushions** that develop in the lengthening outflow tract as second heart field–derived myocardium is added. These endocardial cushions can be discerned into proximal (conal) and distal (truncal) parts. The proximal cushions fuse to form the outflow septum and the wall of the conus portion of both ventricles. The proximal outflow tract septum (conal septum) eventually becomes muscularized through a process referred to as **myocardialization**, whereby the cushion cells are replaced by invading myocardial cells. In trisomy 16 mice (a model for **Down syndrome** in humans), this myocardialization fails, increasing the incidence of outflow tract–related septal defects, which are common in patients with Down syndrome.

The rotation process of the outflow tract and its cushion tissue is necessary for proper alignment of the aorta and pulmonary trunk with their respective ventricles. This realignment has been proposed to develop from the asymmetric addition of second heart field–derived myocardium at the arterial end, resulting in anterior rotation of the pulmonary orifice and trunk in front of the aorta with the aortic orifice remaining in close apposition with the left AV canal. As a consequence, the left and right ventricular outflow tracts and eventually the aorta and pulmonary trunk are twisted around each other in a helical arrangement (Fig. 12-28*D*)—an arrangement that is still obvious in the adult. In addition to the outflow tract septum, a mesenchymal wedge of tissue develops between the fourth and sixth aortic arch vessels (separating the future systemic and pulmonary circuits) in the roof of the aortic sac. This condensed wedge of neural crest–derived mesenchymal tissue forms the **aorticopulmonary septum** (Fig. 12-29), which extends toward the developing outflow tract cushion swellings and fuses with them. Subsequent fusion of the paired outflow tract cushions then proceeds proximally (upstream of the blood flow), partitioning the distal part first and then the proximal outflow tract portion.

Separation of the right and left ventricles is completed when the muscular ventricular septum fuses with the outflow tract septum and the ventricular side of the atrioventricular septum. Development of this **membranous** part of the **ventricular septum** normally occurs between weeks five and eight. Failure of complete fusion results in a ventricular septal defect (see the following "In the Clinic" entitled "Common Heart Malformations: Ventricular Septal Defects").

DEVELOPMENT OF SEMILUNAR VALVES

Animation 12-11: Development of Semilunar Valves. Animations are available online at StudentConsult.

During the formation of the outflow tract septum, two additional smaller cushion tissues form in opposite quadrants of the distal outflow tract called the **intercalated cushion tissue** (Fig. 12-30). The two major outflow tract cushions together with the lateral intercalated cushions are excavated to form cavities at the origin of the future ascending aorta and pulmonary artery. These cavities and the intervening tissue serve as the primordia for the **semilunar valves** and **semilunar sinuses**. Recent studies in mice show that semilunar valve leaflets are mainly of endocardium-derived cushion tissue origin, with some contribution by neural crest cells and possibly epicardial cells. Development of the semilunar valves is complete by nine weeks in humans.

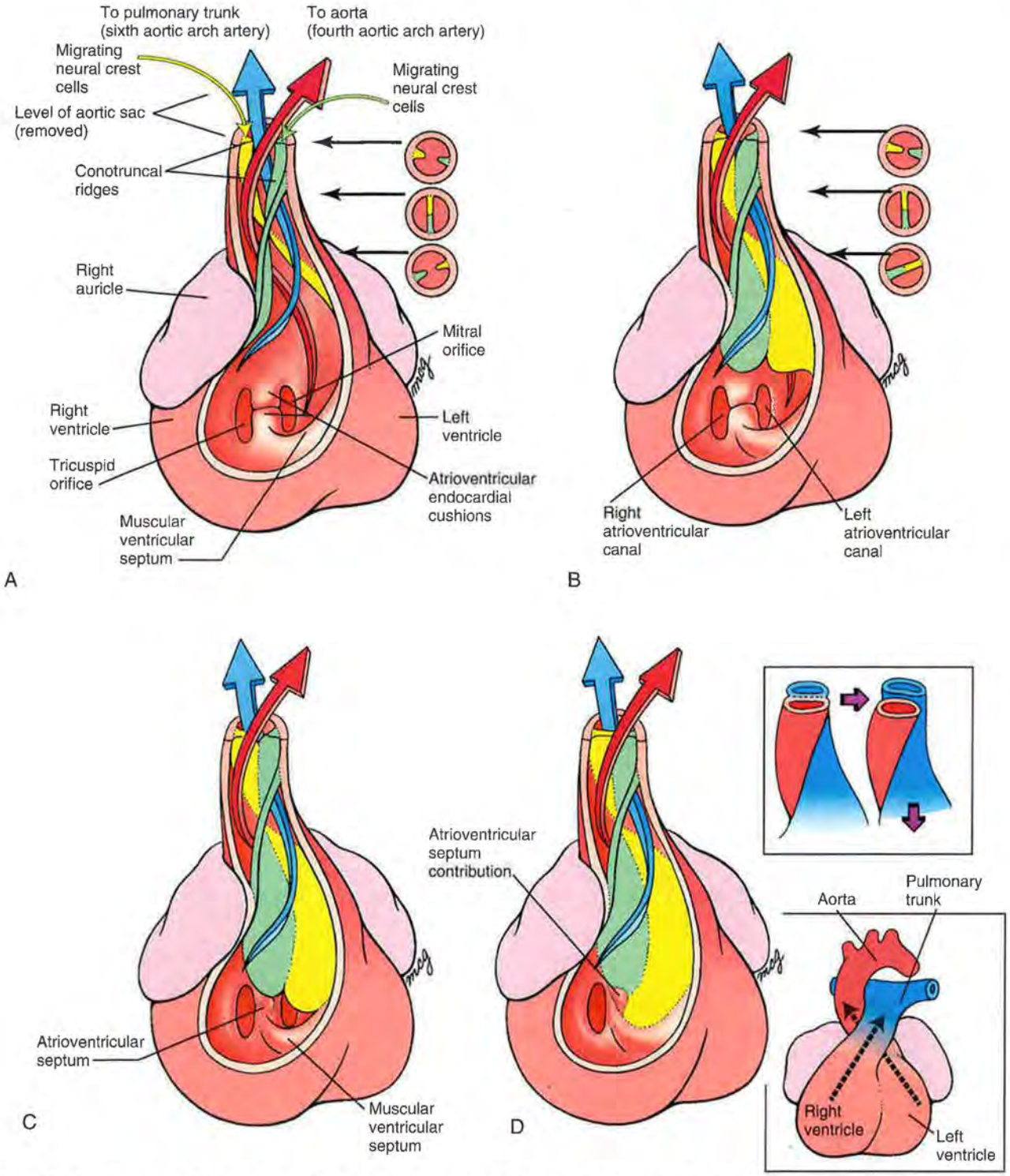

Figure 12-28. Septation of the cardiac outflow tract and completion of ventricular separation. Right oblique view. The cranial-lateral wall of the right ventricle has been removed to show the interior of the right ventricular chamber and the presumptive outflow tracts of both ventricles. *A, B,* Starting in the fifth week, the right and left conotruncal ridges grow out from the walls of the common outflow tract. These swellings are populated by endocardial and neural crest cell–derived cushion cells and develop in a spiraling configuration. They fuse with one another in a cranial-to-caudal direction, forming the conotruncal septum, which separates the aortic and pulmonary outflow tracts. The circular structures to the right of the developing outflow tract illustrate drawings of cross sections at three proximodistal levels. *C, D,* By the ninth week, the caudal end of the conotruncal septum has reached the level of the muscular portion of the ventricular septum and the atrioventricular septum. Here it fuses with these others to complete the ventricular septum.

In the Research Lab

Neural Crest Cell Contribution to Outflow Tract Septation

The importance of neural crest cells in septation of the heart was first shown in ablation studies performed in chick embryos about thirty years ago. If the progenitors of cardiac neural crest cells are removed from embryos before neural crest cells begin to migrate, cardiac looping is abnormal and outflow tract septation is incomplete. Ablation of cardiac neural crest cells causes persistent truncus arteriosus, tricuspid stenosis, ventricular septal defects, transposition of the great arteries, double-outlet right ventricle, and tetralogy of Fallot (see the following "In the Clinic" entitled "Common Heart Malformations: Tetralogy of Fallot"). Moreover, many structural cardiovascular congenital defects involve anomalies of the cardiac neural crest lineage. Further evidence for a role of neural crest cells in heart development is found in the frequent association of these cardiac anomalies with defects in development of the pharyngeal arch structures—through which the cardiac neural crest cells normally migrate. Birth defects in humans involving both the outflow tract and pharyngeal arches include **CHARGE syndrome** (coloboma of the eye, heart defects, atresia of the choanae, retarded growth and development, genital and urinary anomalies, and ear anomalies and hearing loss), **fetal alcohol syndrome**, and **22q11.2 deletion syndrome** (also known as **DiGeorge** or **velocardiofacial syndrome**; these syndromes are covered later and in Chapter 17).

Neural crest cells contributing to the outflow tract and the aorticopulmonary septum are derived from a specific level of the future rhombencephalon and are often referred to as **cardiac neural crest cells** (Fig. 12-31). Both cell-tracing studies using quail-chick transplantation chimeras and transgenic reporter mice (both experimental approaches are covered in Chapter 5) have revealed that not only do neural crest cells invade the outflow tract endocardial cushions, but a subset invade and localize adjacent to the ventricular septum and atrioventricular canal, with some also entering the venous inflow tract. Moreover, evidence suggests that after a time, these neural crest cells undergo apoptosis. What their role is in these regions is unclear, but it may relate to the remodeling that is required to realign the atrioventricular canal and the myocardialization processes in the proximal outflow tract region and in the inflow region. In addition to contributing to the connective tissue and smooth muscle of the distal outflow tract, aorticopulmonary septum, and wall of the aorta and pulmonary trunk, neural crest cells give rise to the parasympathetic postganglionic neurons of the heart (the cardiac ganglia).

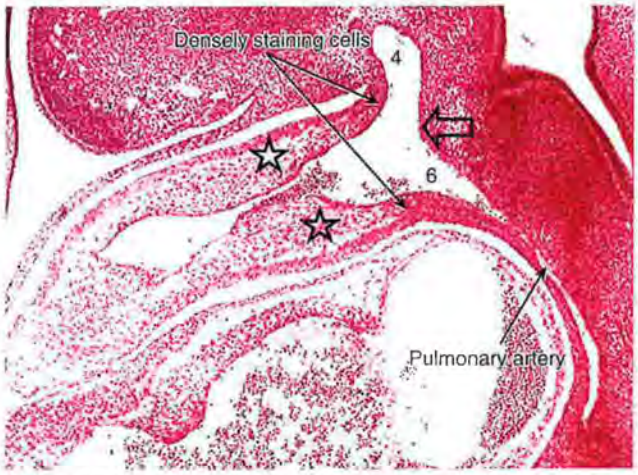

Figure 12-29. Division of the outflow tract in the human embryo. The developing aorticopulmonary septum (indicated by the open arrow) develops between the fourth (labeled 4) and sixth (labeled 6) aortic arch vessels in the roof of the aortic sac and extends toward the fusing outflow tract cushions (indicated by the stars). The densely stained cells include migrating neural crest cells.

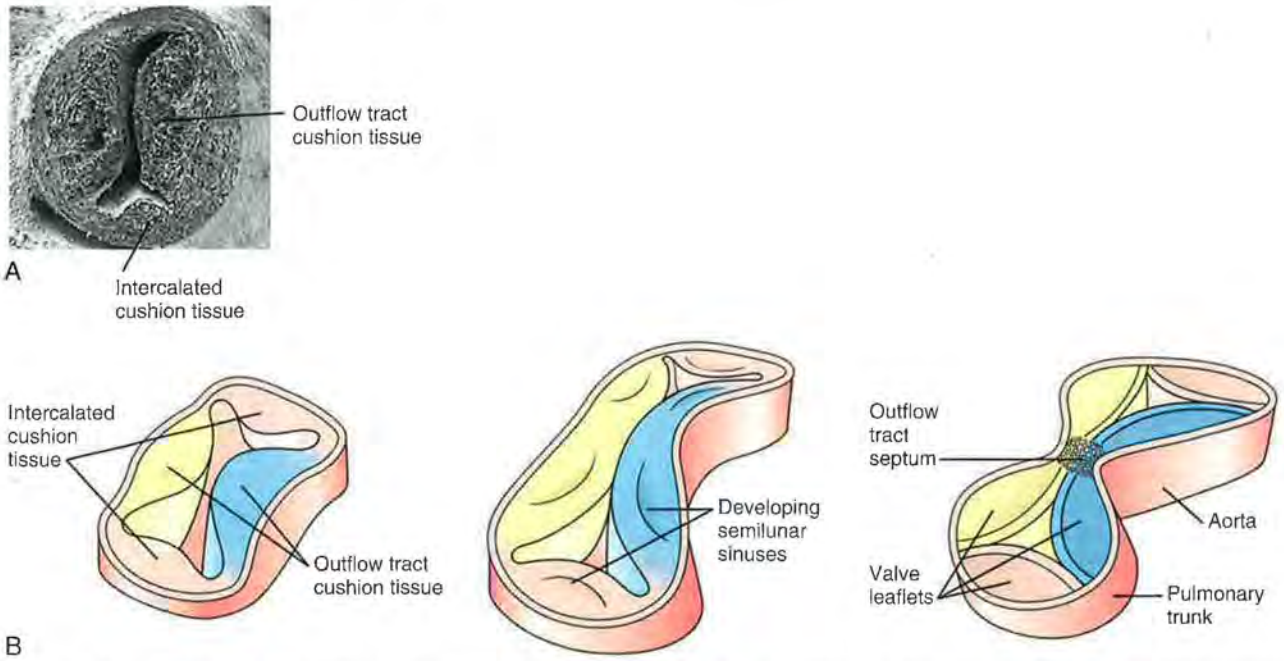

Figure 12-30. Formation of the semilunar valves. *A, B,* During formation of the outflow tract septum, two smaller and shorter intercalated cushion tissues form in the opposite quadrants. At the site of the aortic and pulmonary ostia, this new cushion tissue is excavated and remodeled within the wall of each new vessel to form three cavities. These cavities and the intervening tissue are subsequently remodeled to form the valve sinuses and semilunar valves.

As mentioned earlier, the loss of neural crest cell–derived mesenchymal cells in the outflow tract leads directly to cardiovascular defects. Loss of neural crest cell–derived mesenchyme in the heart can stem from faulty neural crest cell formation, migration, or proliferation. Perturbation of neural crest cell formation, migration, and differentiation results in hypoplasia of the neural crest and an inadequate number of neural crest–derived mesenchymal cells reaching the heart. Several genes have been shown to play important roles in maintaining proper cardiac neural crest cell number and migration. Splotch mice, characterized by a mutation of Pax3, have a reduced number of neural crest cells reaching the pharyngeal arches and entering the outflow tract. The phenotype of these mice resembles that of embryos in which neural crest cells are ablated, including persistent truncus arteriosus and ventricular septal defects. These heart defects, but not the axial defects associated with Splotch mice, are rescued using promoters and enhancers that drive initial neural crest cell–specific Pax3 expression in transgenic Splotch mice.

Double retinoic acid receptor knockout animals (e.g., RARα1 and all RARβ1-3) display intrinsic defects of the myocardium, but they also exhibit anomalies of heart development similar to those produced by ablation of neural crest cells. Lineage-tracing studies of neural crest cells in RARα1/RARβ-deficient mice suggest that neural crest cells themselves do not respond directly to retinoic acid, but rather that the effects on cardiac neural crest cells is indirect. Other important molecules for directing or enabling cardiac neural crest cell migration are semaphorins, a family of secreted molecules important in guiding axons, as well as in directing neural crest cell migration. Sema3C and their receptors, complexes of plexins and neuropilins, are important in targeting cardiac neural crest cells into the pharyngeal arches and outflow tract, as mice lacking Sema3C and neuropilin exhibit persistent truncus arteriosus and great vessel defects.

Endothelins and their converting enzymes and receptors also have important roles in cardiac neural crest development. Knocking out endothelin receptors or their converting enzymes in mice leads to several neural crest cell–related defects, including ventricular septal defects, defects resembling DiGeorge syndrome, pharyngeal arch artery anomalies (covered in Chapter 13), and enteric nervous system defects (covered in Chapter 14), which may be a consequence of neural crest hypoplasia rather than a defect in neural crest migration. In mice in which the Tgfβ type II receptor is knocked out specifically in neural crest cells, cardiovascular defects occur that resemble those seen in DiGeorge syndrome. In this case, migration of neural crest cells into the outflow tract seems normal. However, their subsequent differentiation into smooth muscle cells and connective tissue fails, resulting in persistent truncus arteriosus and ventricular septal defects.

Some Heart Defects May Be Related to Interactions Between Second Heart Field and Neural Crest Cells

As stated earlier, if cardiac neural crest cells are removed from experimental animals before they migrate, several outflow tract and septal defects occur. However, these embryos exhibit other signs of abnormal cardiac development, including cardiac looping defects and early contractility defects, well before the stage when neural crest cells begin to invade the heart. As mentioned earlier, point mutations in Tbx1 lead to heart defects, such as persistent truncus arteriosus and tetralogy of Fallot. Tbx1 is expressed in the endoderm and mesoderm of the second heart field at the arterial pole, and Tbx1 deficiency leads to decreased levels of Fgf8 at that region. Recent studies in chick and mouse embryos suggest that specific levels of Fgf8 are required for proper second heart field cell proliferation. Ablation of neural crest cells increases Fgf8 levels in the second heart field, resulting in outflow tract defects that can be rectified by adding Fgf8 blocking antibodies in chick embryos. Notch signaling also plays a critical role, as loss of notch signaling results in abnormal neural crest migration, alters Fgf8 signaling in the second heart field, and causes outflow tract and great vessel defects. Therefore, it seems that neural crest cells are required for maintaining specific levels of Fgf8 within the second heart field—levels necessary for proper outflow tract lengthening, cardiac looping, and realignment. Neural crest cells may also regulate the expression of several other genes within the second heart field, including goosecoid, Dlx2 and Dlx3, and Hand2. Therefore, abnormal development of cardiac neural crest cells can lead to aberrant heart development by means other than loss of neural crest cell–derived cushion cells within the heart.

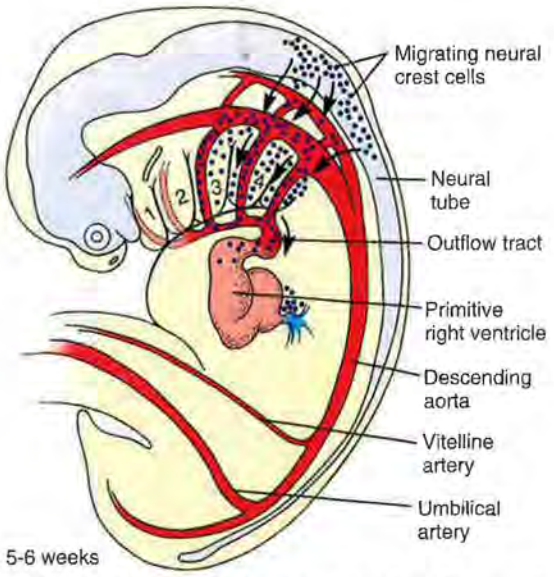

Figure 12-31. Source and migration route of cardiac neural crest cells. Neural crest cells migrate from the hindbrain through pharyngeal arches three, four, and six and then invade the outflow tract and contribute mesenchymal cells to the outflow tract septum. Some neural crest cells also enter the venous inflow region.

DEVELOPMENT OF PACEMAKER AND CONDUCTION SYSTEM

The heart is one of the few organs that has to function almost as soon as it forms. The rhythmic waves of electrical depolarization (action potentials) that trigger the myocardium to contract arise spontaneously in the cardiac muscle itself and spread from cell to cell. The sympathetic and parasympathetic neural input to the heart that arises later in development modifies the heart rate but does not initiate contraction. Cardiomyocytes removed from the primary heart tube and grown in tissue culture will begin to beat in unison if they become connected to one another, and studies with voltage-sensitive dyes indicate that cardiomyocytes may begin to produce rhythmic electrical activity even before the two early endocardial tubes have fused.

Regulation of calcium levels and excitation-contraction coupling are essential for contraction of the first heartbeat. In mouse embryos lacking the sodium-calcium exchanger, Ncx1, the primary heart tube fails to beat, and the embryos are nonviable. In a normally functioning mature heart, the beat is initiated in the **sinoatrial (SA) node** (the **pacemaker**), which has a faster rate of spontaneous depolarization than the rest of the myocardium. Moreover, depolarization spreads from the SA node to the

rest of the heart along specialized **conduction pathways** that control the timing of contraction of various regions of the myocardium, ensuring that the chambers contract efficiently and in the right sequence.

In the primary heart tube, the cardiomyocytes begin to contract asynchronously. Pacemaker activity starts from a transient left SA nodal region that is repositioned to a right SA node, found at the borderline of the entrance to the right common cardinal vein. These cells, derived from the posterior part of the second heart field, eventually differentiate to form the contractile, pacemaking component of the distinct ovoid SA node located at the transition of the superior caval vein and right atrium.

Soon after development of the SA node, cells within the atrioventricular junction adjacent to the endocardial cushion begin to form a secondary pacemaker center, the **atrioventricular (AV) node**, which regulates conduction of impulses from the atrium to the ventricles and coordinates the contraction of the two ventricles. The main conduction pathway between the SA node and the AV node runs through the terminal crest, although controversy continues regarding what internodal pathways do exist. Development of the AV node is accompanied by the formation of a bundle of specialized conducting cells, the **bundle of His**, which sends one branch (the left bundle branch) over the surface of the left side of the ventricular septum and another branch (the right bundle) along the right ventricular septal surface and into the moderator band. This conduction pathway must be carefully avoided during the repair of ventricular septal defects. Branches of **Purkinje fibers** spreading out from the right and left bundle branches then deliver the depolarization signal to the rest of the ventricular myocardium.

The detailed ontogeny of the cardiac conduction system is unclear. However, most of the conduction pathway arises from cardiogenic mesoderm and cardiomyocytes. The myocardial cells of the conducting system are in principle contractile, but they differentiate into cells specialized for generating and conducting action potentials responsible for mediating rhythmic and wave-like contraction of the heart. Expression of Tbx2 and Tbx3, important in patterning of non-chamber myocardium (e.g., atrioventricular canal and outflow tract myocardium) is required for proper development of the conduction system (Fig. 12-32). Subsequent expression of endothelin and neuregulin signaling from the overlying endocardium and coronary endothelium plays a major role in mediating the differentiation of cells constituting the central conducting and Purkinje system. Nkx2.5 expression levels increase during the development of the conducting system and mediate the expression of gap junctional proteins essential for coupling the specialized cardiomyocytes. Mice haploinsufficient for Nkx2.5 exhibit severe hypoplasia of the conducting system. In humans, mutations in NKX2.5 are associated with structural anomalies of the conduction system and progressive disease of the atrioventricular conduction system. Understanding development of this network is important, as many adults experience arrhythmias, with some anomalies being associated with mutations in developmental control genes having key roles in heart development. A better understanding of the embryonic development of the conducting system may shed light on the etiological basis of congenital arrhythmias.

DEVELOPMENT OF EPICARDIUM AND CORONARY VASCULATURE

The progenitor of the epicardium, the **proepicardial organ**, consists of a special group of splanchnic mesodermal cells formed at the caudal dorsal mesocardium/septum

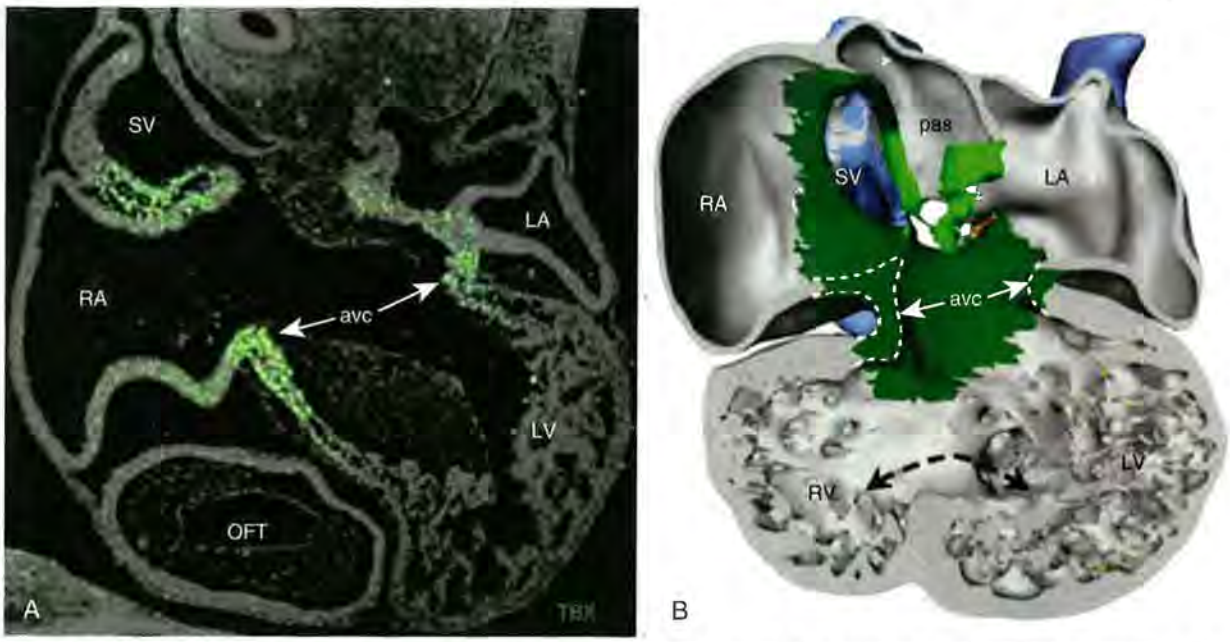

Figure 12-32. Expression pattern of TBX3 in the heart of a five-week-old human embryo. *A*, Localization of TBX3 mRNA expression in the atrioventricular region and sinus venous by situ hybridization. *B*, Three-dimensional reconstruction of TBX3 expression showing predominant expression in regions where the pacemaker and the conducting system develop. Dashed arrow indicates the location of the interventricular foramen. avc, Atrioventricular cushion; LA, left atrium; LV, left ventricle; OFT, outflow tract; pas, primary atrial septum (septum primum); RA, right atrium; RV, right ventricle; SV, sinus venosus.

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transversum junction (Fig. 12-33*A*; see also Fig. 12-19*B*). Proepicardial cells express both Wilm's tumor protein-1 (Wt1) and Tbx18. With the exception of epicardial precursor cells migrating from the cranial dorsal mesocardium to cover a portion of the outflow tract, proepicardial organ cells migrate as an epithelial sheet of cells over the entire myocardial surface (Fig. 12-33*B*). Once covering the surface of the myocardium, the epicardial epithelium deposits and assembles an extracellular matrix between the epicardial epithelium and myocardium. This is followed by an

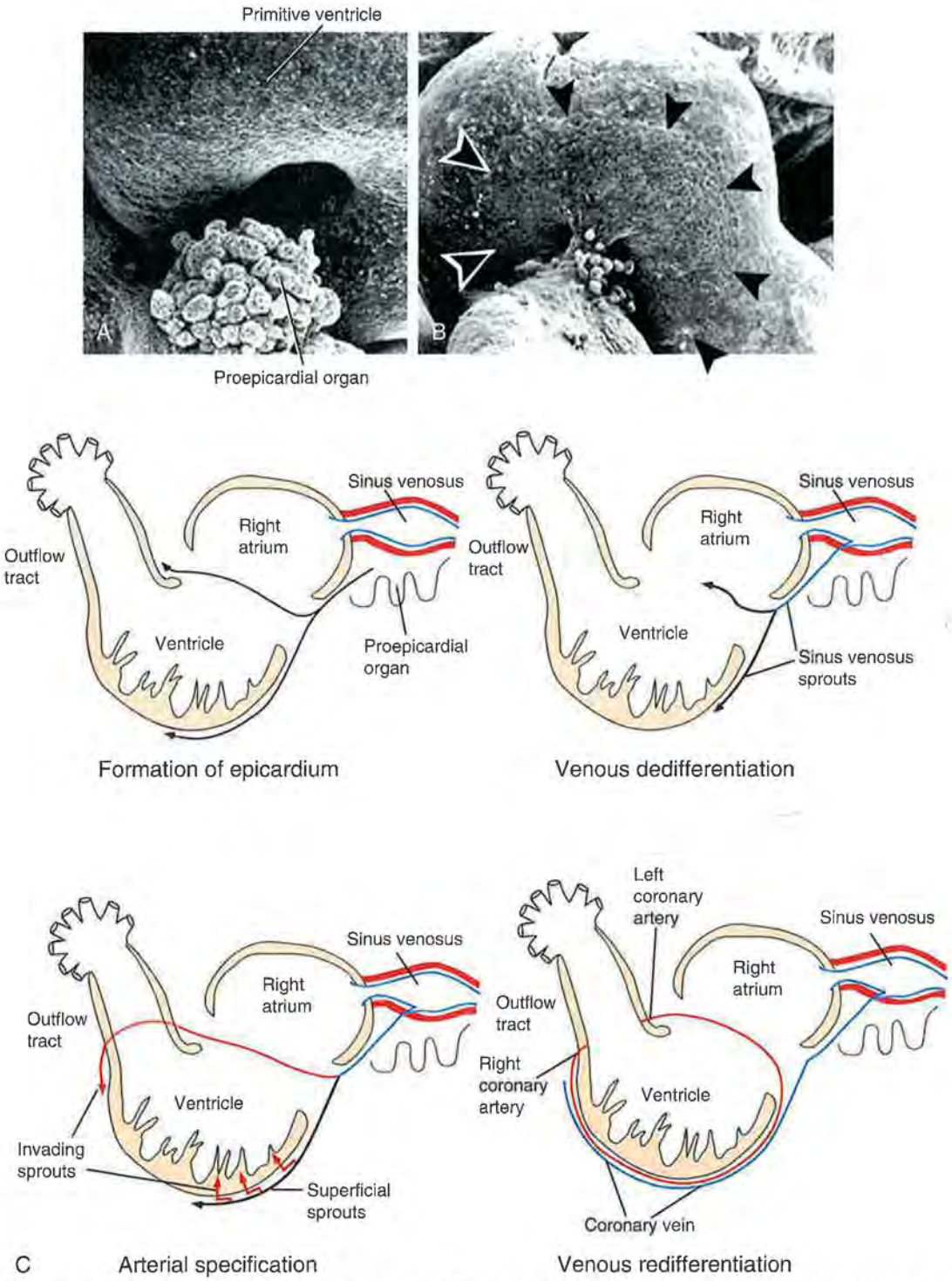

Figure 12-33. Formation of the epicardium and coronary vessels. *A, B,* The epicardium is formed from migrating cells derived from the proepicardial organ found in the region of the sinus venosus. As indicated in these scanning electron micrographs, these cells migrate over and cover the entire myocardium (arrowheads), eventually forming the epicardium. *C,* The endothelial precursors for the coronary vessels arise from sprouts of the sinus venosus. These sprouts lose their venous phenotype, migrate through the epicardium, and also invade the myocardium. The invading sprouts form endothelial cells expressing arterial markers and eventually form the right and left coronary arterial endothelia. The superficial sprouts form the coronary venous endothelium. Epicardium-derived cells form the coronary vascular smooth muscle and cardiac fibroblasts, and a subset also differentiate into cardiomyocytes.

epithelial-to-mesenchymal transformation of the epicardial epithelium, generating a mesenchymal cell population that invades the subepicardial extracellular matrix in much the same way that endocardium-derived cushion tissue is generated. Recent studies suggest that these **epicardium-derived mesenchymal cells** not only form cardiac fibroblasts and coronary vascular smooth muscle, but that they also differentiate into functional cardiomyocytes that contribute to the muscular ventricular septum and atria, with smaller numbers scattered throughout the ventricles.

Until recently, epicardium-derived mesenchymal cells were thought to provide the progenitor cells for the coronary endothelium. However, studies in mice show that coronary endothelial cells actually arise from angiogenesis (sprouting from preexisting blood vessels; covered in Chapter 13) of the sinus venosus (Fig 12-33C). Venous endothelial precursor cells of the sinus venosus dedifferentiate (lose venous markers) and migrate over and invade the myocardium. These cells differentiate into coronary endothelial cells of arteries and capillaries (express arterial markers), with only a minor contribution of endothelial cells coming from the endocardial lining. The endothelial precursor cells remaining on the surface of the heart redifferentiate into venous endothelial cells. The angiogenic processes leading to formation of the coronary vascular network involve many of the same signaling molecules and regulatory events as occur during blood vessel formation elsewhere in the embryo (covered in Chapter 13). The connection of the developing coronary vasculature to the aorta occurs by invasion of the developing coronary arteries through the wall of the (ascending) aorta. Why normally only two coronary artery trunks form and how they find their way to the future site of the aortic sinuses are still unclear.

In the Research Lab

MicroRNAs as Regulators of Cardiac Development

Studies suggest that microRNAs (miRNAs) play important roles in regulating cardiovascular development and disease. Genomically encoded, miRNAs are transcribed and processed by the nuclear enzymes drosha and dicer to yield mature miRNAs. miRNAs leave the nucleus and are incorporated into the RNA-inducing silencing complex, where they bind to their sequenced-matched target mRNAs and initiate the destruction or inhibit the translation of their target genes. Mice null for dicer in Nkx2.5-expressing cells develop a hypoplastic ventricular myocardium and die of cardiac failure while in utero. Mouse embryos with cardiac targeted loss or overexpression of specific miRNAs develop ventricular septal defects and conduction defects and exhibit abnormal expression of several transcription factors required for normal cardiomyocyte proliferation and development. Deletion of dicer from neural crest cells causes ventricular septal defects, double-outlet right ventricle, and aortic arch defects, as well as loss or hypoplasia of many neural crest–derived structures.

In humans, more than 600 miRNAs have been identified. Recent studies show that coding sequences for several miRNAs are located on chromosome 21. Individuals with Down syndrome overexpress several miRNAs and show corresponding decreases in expression of several target genes thought to be responsible for the Down syndrome phenotype, including congenital craniofacial and cardiac defects. Hence, a better understanding of the regulation of miRNA expression, identification of their targets, and functional consequences of their expression will be necessary for elucidating the etiological mechanisms behind many congenital defects.

In the Clinic

Frequency and Etiology of Cardiovascular Malformations

Congenital cardiovascular malformations account for about 20% of all congenital defects observed in live-born infants. They occur in about 5 to 8 of every 1000 live births, and the percentage in stillborn infants is probably even higher. In addition, the recurrence risk in siblings with isolated heart malformations is 2% to 5%, indicating that heart defects include a genetic contribution.

Neither the cause nor the pathogenesis of most heart defects is completely understood. In fact, it is still unclear what genes and cellular process initiate the heartbeat and how the myriad of extracardiac cell lineages is integrated to give rise to a mature fully functional four-chambered heart. However, progressively more of these defects are being associated with specific genetic errors or environmental teratogens. Overall, about 4% of cardiovascular defects can be ascribed to single-gene mutations, another 6% to chromosomal aberrations such as trisomies, monosomies, or deletions, and 5% to exposure to specific teratogens. The teratogens known to induce heart defects include not only chemicals such as lithium, alcohol, and retinoic acid but also factors associated with certain maternal diseases such as diabetes and rubella (German measles). The etiology of most of the remaining cardiac abnormalities (about 80% to 85%) seems to be **multifactorial**—that is, they stem from the interaction of environmental or outside influences **(epigenetic)** with a poorly defined constellation of the individual's own genetic determinants. Thus, individuals may show very different genetic susceptibilities to the action of a given teratogen. Blood pressure and blood flow, factors unique to the developing cardiovascular system, play important roles in the development of the heart such that perturbations in pressure relationships among the heart chambers and outflow tracts cause malformations. Such perturbations may be brought about by several kinds of primary defects—by abnormal compliance or deformability of the atrial, ventricular, or outflow tract walls or by abnormal expansion or constriction of semilunar valves, ductus arteriosus, and great arteries (covered in Chapter 13). For example, if ejection of blood from the right ventricle is prevented by pulmonary valve atresia, the right ventricle becomes **hypoplastic** and the pulmonary arteries are underdeveloped. If blood flow into the right ventricle from the right atrium is prevented by tricuspid atresia, the right ventricle becomes **hypoplastic** while the left ventricle **hypertrophies** because of the extra workload placed on it to drive blood into the pulmonary circulation through a ventricular septal defect. Excessive interatrial flow can cause a septum secundum defect by enlarging the foramen ovale and eroding septal structures. The resulting increased inflow through the left side of the heart may interfere with the normal formation of the outflow tract septum and prevent development of the membranous ventricular septum.

Common Heart Malformations

Atrial Septal Defects

In about 6 of 10,000 live-born infants, the septum secundum is too short to cover the foramen secundum completely (or the foramen secundum is too large), so that an **atrial septal defect** persists after the septum primum and septum secundum are pressed together at birth (Fig. 12-34). Atrial septal defects cause shunting of blood from the left atrium to the right atrium. Infants

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with this abnormality are generally asymptomatic, but the persistent increase in flow to the right atrium may lead to enlargement of the right atrium and ventricle, resulting in debilitating atrial arrhythmias later in life. Excessive pulmonary blood flow also causes pulmonary hypertension over time, leading to heart failure. Atrial septal defects are most often detected by echocardiography in childhood, and they may warrant closure surgically or by an occluding device to prevent the onset of cardiac hypertrophy and pulmonary hypertension. An atrial septal defect is associated with almost all documented autosomal and sex chromosome aberrations, and it is a common accompaniment of several partial and complete trisomies, including trisomy 21 (Down syndrome).

Persistent Atrioventricular Canal

Persistent atrioventricular canal or **atrioventricular septal defect** arises from failure of the dorsal and ventral endocardial cushions to fuse. Failure of the dorsal and ventral endocardial cushions to fuse can lead to a variety of secondary abnormalities, including atrial septal defects, ventricular septal defects, and malformation of the atrioventricular valves. One physiological consequence of the defect is persistent left-to-right shunting of blood after birth, the magnitude of which depends on the severity of the defect. Pulmonary hypertension and congestive heart failure in infancy are likely if the defect is severe. Atrial septal defects, malformed atrioventricular valves, and absence of a ventricular septum can be corrected surgically.

Ventricular Septal Defects

Ventricular septal defects are some of the most common of all congenital heart malformations, accounting for 25% of all cardiac abnormalities documented in live-born infants and occurring as isolated defects in 12 of 10,000 births (Fig. 12-35). The prevalence of this defect seems to be increasing—a statistic that may represent an actual increase in incidence or may reflect the application of better diagnostic methods. A ventricular septal defect can arise from several causes: (1) deficient development of the proximal outflow tract cushions, (2) failure of the muscular and membranous ventricular septal components to fuse, (3) failure of the dorsal and ventral endocardial cushions to fuse (atrioventricular septal defect), (4) insufficient development of the muscular ventricular septum, and (5) altered hemodynamics. Whatever the origin of a ventricular septal defect, its most serious consequence is the left-to-right shunting of blood and the consequent increased blood flow to the pulmonary circulation. In some cases, a ventricular septal defect closes spontaneously during infancy. If it persists and causes a hemodynamic problem, it can be repaired surgically or percutaneously with a device.

Atrioventricular Valve Defects

Atrioventricular valve defects arise from errors in the remodeling necessary for the formation of valve leaflets, chordae tendineae, and papillary muscles from the endocardial cushion tissue and ventricular myocardium. The pathogenesis of **atrioventricular valve atresia**, in which the valve orifice is completely obliterated, is not understood. In **tricuspid valve atresia**, the right atrium is cut off from the right ventricle as the result of abnormal development of the tricuspid valve. Tricuspid valve atresia could be due to an abnormal expansion of the right side of the atrioventricular canal so that a normal inflow tract of the right ventricle is not established. Alternatively, the tricuspid orifice may be only partly connected to the right ventricle, resulting in a straddling tricuspid valve with chordae attached to both ventricles. If the tricuspid orifice remains in its entirety above the left ventricle, a **double-inlet left ventricle** forms. Regardless, the result is that right atrial blood must be shunted to the left atrium through a **persistent foramen ovale**. Moreover, most of the blood reaching the pulmonary arteries does so by taking a roundabout route through a ventricular septal defect and/or via the aorta and a **patent (persistent) ductus arteriosus**. The ductus arteriosus is a connection between the aorta and the pulmonary trunk that normally closes soon after birth (covered in Chapter 13). As a consequence, the heart is functionally a univentricular heart, as the circulation is driven solely by the left ventricle. Hence, the right ventricle is **hypoplastic** while the left ventricle enlarges (i.e., **hypertrophies**). Over time, this leads to cardiac failure.

Malalignment of the outflow tract may lead to a ventricle having a double outlet (having both the aorta and the pulmonary artery). In **double-outlet right ventricle**, both aortic and pulmonary outflow tracts connect to the right ventricle, and this malformation is almost always accompanied by a ventricular septal defect. All arterial blood flow leaves from the right ventricle, and oxygenated blood is mixed with deoxygenated blood within the right ventricle. Symptoms show up within days after birth and include **cyanosis** (due to inadequate oxygenation of the blood), heart **murmur**,

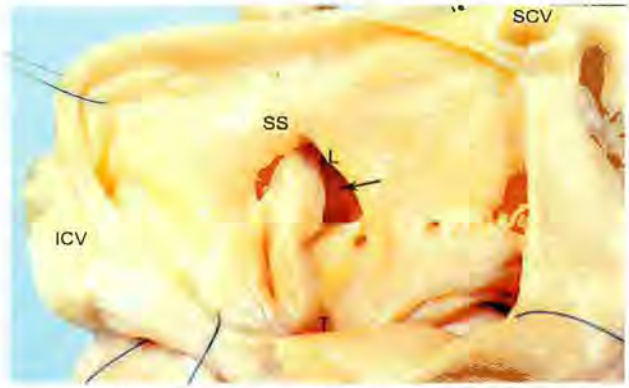

Figure 12-34. Heart with an atrial septal defect (arrow) from a human infant. The foramen secundum and foramen ovale in this heart overlap abnormally; therefore, the foramen ovale could not close at birth, resulting in continued mixing of right and left atrial blood after birth. ICV, Inferior caval vein; L, limbus of the foramen ovale; SCV, superior caval vein; SS, septum secundum; T, tricuspid orifice.

Figure 12-35. Typical ventricular septal defect (VSD) in a heart from a human infant. Failure of the membranous ventricular septum to fuse with the upper ridge of the muscular ventricular septum in this heart has resulted in a ventricular septal defect (arrow). OS, Outflow tract septum; P, pulmonary outlet; RV, right ventricle; T tricuspid orifice.

breathlessness, and (later) poor weight gain. The incidence of this malformation is approximately 1 in 3000 births, and it can be corrected surgically.

Semilunar Valve Stenosis

Semilunar valve stenosis involves stenosis of the aortic or pulmonary valve. **Aortic valve stenosis** leads to **hypertrophy** of the left ventricle, pulmonary hypertension, and eventually cardiac failure. It can be congenital (usually the case if symptoms appear before age 30), the result of an infection (such as rheumatic fever), or degenerative (a consequence of aging). Collectively, the incidence is 1% to 2% of the population, with greater frequency in males (4:1 male-to-female ratio). Congenital valve stenosis is likely caused by an error in cavitation and remodeling within the distal outflow tract cushion tissue responsible for forming the aortic and pulmonary semilunar valves. This can lead to a **bicommissural aortic valve** (having two rather than three leaflets). A bicommissural valve can be asymptomatic or stenotic from infancy or may become stenotic over time, often as the result of calcification.

Septation Defects of Outflow Tract

A variety of malformations resulting from errors in septation of the outflow tract may be caused by abnormal neural crest cell development. In about 1 of 10,000 live-born infants, the outflow tract septa do not form at all, resulting in a **persistent truncus arteriosus** (Fig. 12-36*A, B*). This malformation necessarily includes a **ventricular septal defect**. The result is

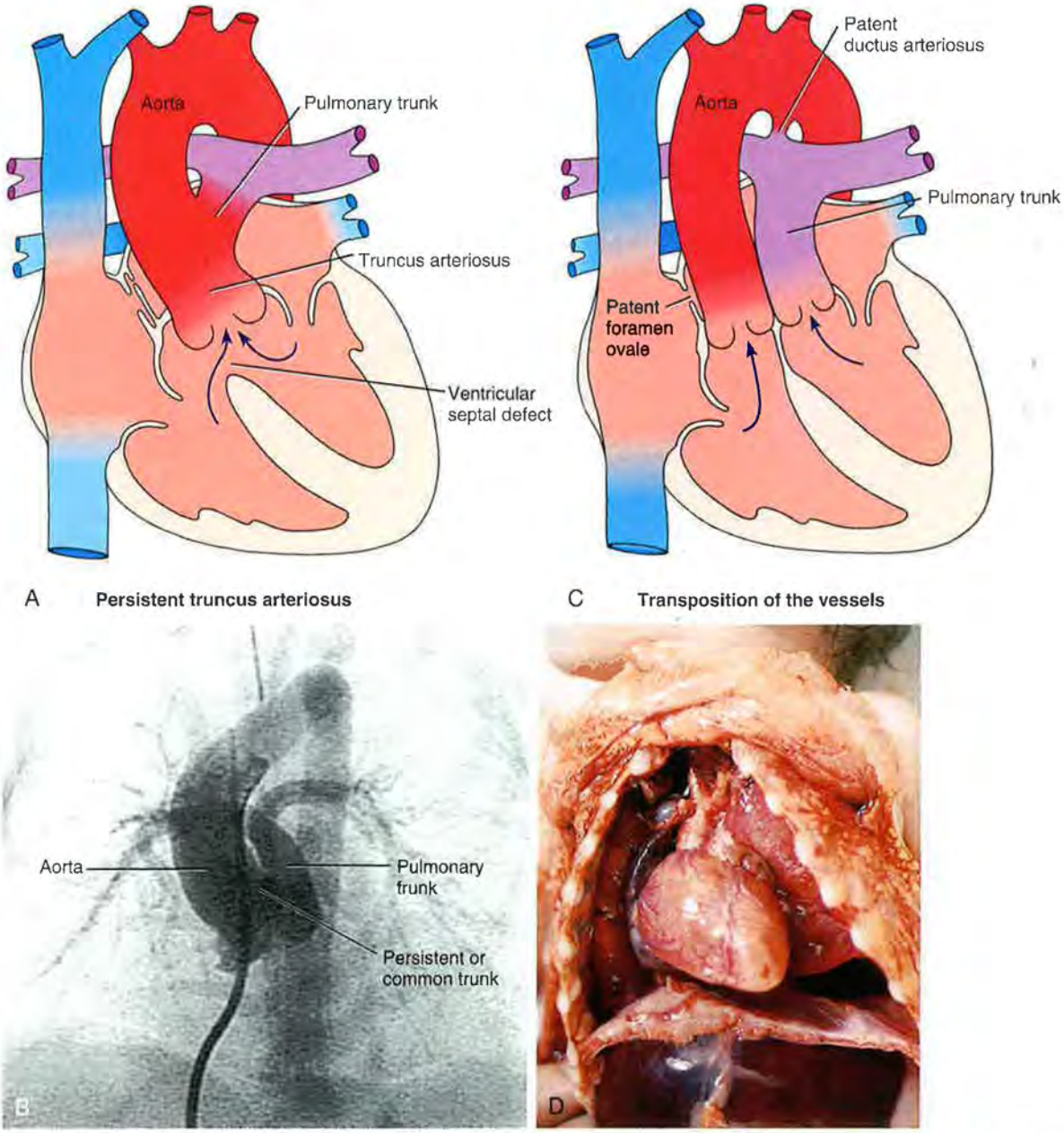

Figure 12-36. Outflow tract anomalies. *A, B,* Persistent truncus arteriosus (shown in an angiogram in *B*). Incomplete separation of aortic and pulmonary outflow tracts accompanies a ventricular septal defect when the outflow tract septum fails to form. *C, D,* Transposition of the great arteries occurs when the ventricular outflow vessels run in parallel and do not connect to their proper outflow vessel.

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that blood from the two sides of the heart mixes in the common outflow tract, mainly in left-to-right shunting toward the pulmonary side, leading to pulmonary hypertension. Left untreated, infants with this defect usually die within the first 2 years. Surgical correction is possible and involves repairing the ventricular septal defect and implanting a valved prosthetic shunt between the right ventricle and the pulmonary arteries.

In about 5 of 10,000 live-born infants, the outflow tract septa develop but the vessels are positioned in parallel and do not connect to the proper outflow vessel. New data suggest that this results from a disturbance in the contribution of the second heart field to the outflow tract myocardium. The result is **transposition of the great vessels**, in which the left ventricle pumps blood into the pulmonary circulation and the right ventricle empties into the systemic circulation (Fig. 12-36*C, D*). Transposition of the great vessels is often fatal unless the ductus arteriosus remains patent, or it is accompanied by intrinsic atrial or ventricular septal defects or by defects introduced surgically (to establish an interatrial communication), allowing the deoxygenated systemic and the newly oxygenated pulmonary blood to mix. Transposition can be surgically corrected with a favorable prognosis. Nevertheless, it is the leading cause of death in infants with cyanotic heart disease younger than 1 year of age.

Tetralogy of Fallot

Many cardiac defects occur together more often than in isolation. In some cases, such associated defects are actually components of the same malformation—as, for example, a ventricular septal defect is a necessary consequence of persistent truncus arteriosus. In other cases, a primary malformation sets off a cascade of effects that lead to other malformations. An example is the pathogenesis of **tetralogy of Fallot**, a syndrome referred to as *maladie bleue* by Etienne-Louis Arthur Fallot in 1888 (Fig. 12-37). Fallot used the term *tetralogy* to refer to the four classic malformations in this syndrome: (1) **pulmonary trunk stenosis**, (2) **ventricular septal defect**, (3) rightward displacement of the aorta (sometimes called **overriding aorta**), and (4) **right ventricular hypertrophy**. The primary defect is unequal division of the outflow tract, favoring the aorta, with malalignment of the outlet septum with respect to the right and left ventricles. All of these defects conspire to raise the blood pressure in the right ventricle, resulting in progressive right ventricular hypertrophy. Tetralogy of Fallot is the most common cyanotic congenital heart malformation, occurring in approximately 1 of 1000 live-born infants. The condition may be corrected surgically by relieving the obstruction of the pulmonary trunk and repairing the ventricular septal defect.

Known Genetic Causes of Heart Malformations

Based on genetic studies in families, many cardiac malformations have been ascribed to single-gene mutations, with continuing progress in identifying more through animal studies and human genetic linkage studies. However, to date only a few have been found that are non–syndrome-associated gene mutations occurring in so-called isolated heart defects. One of the earliest acting of these mutations occurs in NKX2.5. This gene plays an important role in specification of the early cardiogenic field, but it is also involved in several subsequent cardiac morphogenic events. Mutations in NKX2.5 in humans are associated with atrial septal defects and defects in the conduction system. Mutations in GATA4 have also been found in the human population. These mutations alter the transcriptional activity of GATA4 and its interaction with other gene products important in cardiac development, including NKX2.5 and TBX5. Mutations in GATA4 have been linked to atrial septal defects and pulmonary valve stenosis. Mutations in TBX20, a transcription factor important in chamber specification (covered earlier), are linked to atrial and ventricular septal defects, valve defects, and abnormal chamber growth. Mutations in CYSTEINE-RICH PROTEIN WITH EGF-LIKE DOMAINS (CRELD1; a cell adhesion molecule) have been found in patients with atrioventricular septal defects.

A number of specific gene mutations have also been identified in syndromes that contain heart defects as a consistent finding. Mutations have been found in various genes causing laterality and cardiac looping defects. Mutations in genes encoding axonemal DYNEINS are found in patients with **Kartagener syndrome** (covered in Chapters 3 and 11). **Randomized laterality** and **visceroatrial heterotaxy** occur in patients with mutations in NODAL, LEFTY1, LEFTY2, CRYPTIC, and ACVR2B (an ACTIVIN receptor). Patients with **LEOPARD syndrome** or **Noonan syndrome** exhibit pulmonary trunk stenosis, atrioventricular septal defects, and conduction anomalies, as well as over-

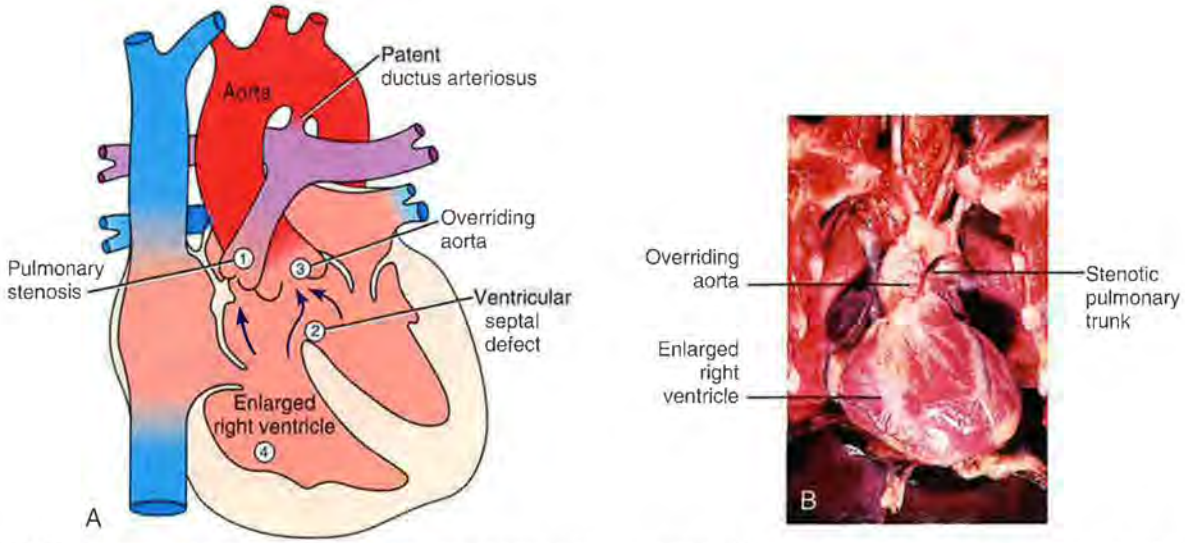

Figure 12-37. Tetralogy of Fallot. *A,* Classically, tetralogy of Fallot is characterized by (1) stenosis (narrowing) of the pulmonary trunk, (2) ventricular septal defect, (3) overriding aorta, and (4) an enlarged right ventricle. A patent ductus arteriosus is also present. *B,* The enlarged right ventricle and overriding aorta are obvious in this case of tetralogy of Fallot in a heart from a human infant.

lapping craniofacial and skeletal anomalies that can be caused by different mutations in the PTPN11 gene. This gene encodes an SHP2 protein, a non-receptor tyrosine phosphatase involved in intracellular signal transduction (Noonan syndrome is also covered in Chapter 13). Deletion or mutations in the JAGGED1 gene (a gene encoding a ligand for NOTCH signaling) or NOTCH1/NOTCH2 (genes encoding for NOTCH receptors) is responsible for **Alagille syndrome** (covered in Chapter 3, 5, 13, and 14), and 70% to 95% of these patients exhibit heart defects, including stenosis of the pulmonary and aortic arteries or valves, septal defects, and tetralogy of Fallot. However, it is unclear whether Alagille syndrome is caused by abnormalities in epithelial-to-mesenchymal transformation of the endocardium or in later valve development. Mutations of the CHD7 (CHROMODOMAIN HELICASE DNA-BINDING PROTEIN 7) gene on human chromosome 8 have been found in 60% of patients with **CHARGE syndrome** (incidence 1 of 9000 to 10,000; also covered in Chapters 4 and 17), and 75% of these patients exhibit heart defects. Studies in human embryos show that neural crest cell–derived mesenchyme is one of the primary tissues expressing this gene.

Most of the 250,000 individuals who suffer sudden death each year in the United States die of **cardiac arrhythmias**. One inborn cause of arrhythmias is **long QT syndrome**, characterized by prolongation of depolarization (Q) and repolarization (T) intervals diagnosed by electrocardiogram (ECG or EKG). Long QT syndrome predisposes affected individuals to **syncope** (loss of consciousness) and sudden death. It is no surprise that genetic disruptions underlying this autosomal dominant disease include mutations in KVLQT1, HERG, SCN5A, and other genes that encode **cardiac ion channels**.

22Q11.2 Deletions and Heart Malformations

Patients with **22q11.2 deletion syndrome** (also known as **DiGeorge** and **velocardiofacial syndromes**) exhibit congenital anomalies that place them within the neurocristopathy family of defects (covered in Chapter 4; 22q11.2 deletion syndrome is also covered in Chapters 13 and 17). These defects involve at least one element of abnormal neural crest cell development and manifest congenital heart defects as part of the pathology. These patients have microdeletions within the 22q11.2 region, which occur in 1 of 10,000 to 20,000 live births. Common heart defects are tetralogy of Fallot, interrupted aortic arch (covered in Chapter 13), ventricular septal defects, persistent truncus arteriosus, and vascular rings (covered in Chapter 13). Therefore, presentation of these types of defects should alert the physician to look for the possibility of 22q11.2 deletions and other pathologic conditions that may arise from such deletions. Examination of the genes in this region is underway to determine which may be responsible for the symptoms of these deletions. Links to several genes have been identified, of which TBX1, expressed in the second heart field and adjacent pharyngeal pouch endoderm, is the most likely one. Others include UFD1 (UBIQUITIN FUSION DEGRADATION-1, a gene regulated by HAND2) and HIRA (a gene encoding for a protein that interacts with PAX3). In the case of TBX1, rare mutations have been found in patients with DiGeorge phenotype but lacking a 22q11.2 deletion, suggesting that in some cases, a single gene can cause DiGeorge syndrome. However, in the vast majority of patients, loss of multiple linked 22q11.2 genes is likely responsible.

Embryology in Practice

Caught in the Middle

A previously healthy twenty-year-old man suddenly collapses while running during the final leg of a triathlon. Fortunately, the triathlon emergency medical staff witnesses the event and rushes to his aid. They find him pulseless, and one begins CPR while the other retrieves the automatic cardiac defibrillator (ACD). After several shocks, the man is returned to a normal sinus rhythm and is transferred to the hospital by ambulance.

In the emergency department, the man is conscious, and after some time he is able to answer questions. When asked about his medical history, he states that he "has none," having "never been to a doctor." He describes himself as very healthy and states that he was a competitive runner in school. He denies previous fainting spells, chest pain, palpitations, or shortness of breath. An electrocardiogram shows ST segment changes and elevated cardiac troponins, indicating acute myocardial ischemia.

A cardiac catheterization shows no evidence of atherosclerotic narrowing of his coronary arteries and instead reveals an anomalous **left coronary artery** (LCA) originating from the *right* sinus of Valsalva (i.e., **aortic sinus**) and passing between the pulmonary and aortic trunks, where it is vulnerable to compression during systole, with subsequent reduced myocardial perfusion (Fig. 12-38). This situation can be exacerbated by exercise, the first symptom of which can be sudden cardiac death. The patient is taken to the operating room, where the surgeon is able to reimplant the LCA to the left sinus of Valsalva. He makes a full recovery.

Similar to other vascular structures, the coronary arterial system shows considerable plasticity. In contrast to variation seen in the systemic arteries, variation in the coronary arteries can have serious consequences for the heart muscle. A related condition, anomalous origin of the left coronary artery arising from the pulmonary artery (ALCAPA), can present in infancy, as the LCA in this instance is supplied by poorly oxygenated blood from the right side of the heart and is often hypoperfused as the result of pulmonary steal to the lower-pressure lung vasculature.

The pathogenesis of these conditions is not clearly known, but they might result from abnormal partitioning of the truncus arteriosus or from agenesis or regression of one of the coronary arterial buds.

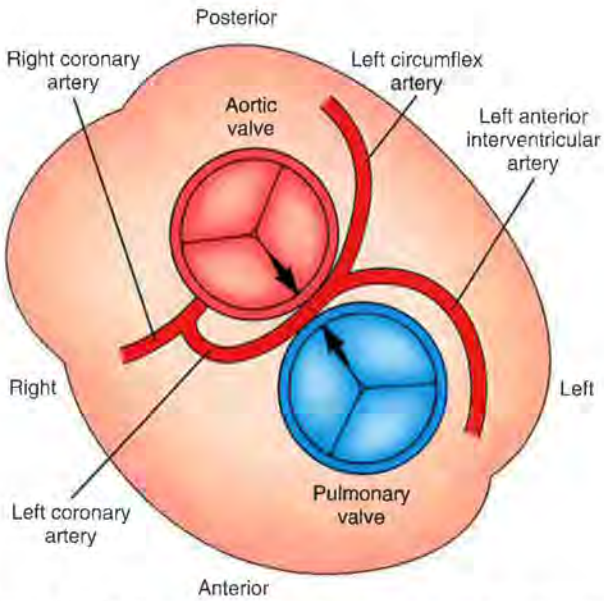

Figure 12-38. Origin of the left coronary artery from the right aortic sinus. Arrows indicate compression of the left coronary artery during systole. The outline of the heart at the level of the aortic and pulmonary valves is indicated.

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